

We claim:

1. A system for monitoring in real time the progress of a nucleic acid amplification reaction, the system comprising:
  - 5 a reaction mixture for carrying out the nucleic acid amplification reaction so that an amplification product is synthesized;
  - a sample interface comprising a fiber optic co-axially disposed with a lens so that an excitation beam transmitted by the fiber optic is focused into a volume of the reaction mixture by the lens;
  - 10 a first fluorescent indicator capable of generating a first fluorescent signal whose intensity is proportional to the amount of an amplification product in the volume of the reaction mixture illuminated by the excitation beam; and
  - a second fluorescent indicator homogeneously distributed throughout the reaction mixture and capable of generating a second fluorescent signal proportional to
  - 15 the volume of reaction mixture illuminated by the excitation beam;wherein the lens of the sample interface collects a portion of the first fluorescent signal and the second fluorescent signal and focuses said portion onto the fiber optic, the fiber optic transmitting said portion to a detection and analysis means.
- 20 2. The system of claim 1 wherein said first fluorescent signal has an intensity and said second fluorescent signal has an intensity, and wherein said detection and analysis means provides a readout comprising a ratio of the intensities of said first fluorescent signal and said second fluorescent signal.
- 25 3. The system of claim 2 wherein said first fluorescent indicator is a complex-forming dye.
4. The system of claim 3 wherein said system comprises a plurality of said sample interfaces, each said fiber optic of said sample interface having a first end co-axially
- 30 disposed with said lens and a second end coupled to a fiber optic multiplexer.
5. The system of claim 3 wherein said reaction mixture is contained in a closed reaction vessel, said lens focusing said excitation beam through a portion of a wall of the closed reaction vessel into said reaction mixture, and said portion of the wall being
- 35 heated so that condensation of components of said reaction mixture thereon is prevented.

6. The system of claim 3 wherein said system comprises a plurality of said first fluorescent indicators each corresponding to a different said amplification product.

5 7. The system of claim 3 wherein said amplification reaction is a polymerase chain reaction.

8. The system of claim 3 wherein said amplification reaction is a ligase chain reaction

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9. The system of claim 2 wherein said amplification reaction is a polymerase chain reaction and wherein said first fluorescent indicator and said second fluorescent indicator are covalently attached to an oligonucleotide having a nucleotide sequence complementary to a portion of a strand of said amplification product, said second fluorescent indicator quenching the fluorescence of said first fluorescent indicator.

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10. The system of claim 9 wherein said system comprises a plurality of said sample interfaces, each said fiber optic of said sample interface having a first end co-axially disposed with said lens and a second end coupled to a fiber optic multiplexer.

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11. The system of claim 9 wherein said reaction mixture is contained in a closed reaction vessel, said lens focusing said excitation beam through a portion of a wall of the closed reaction vessel into said reaction mixture, and said portion of the wall being heated so that condensation of components of said reaction mixture thereon is prevented.

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12. The system of claim 9 wherein said system comprises a plurality of said first fluorescent indicators each corresponding to a different said amplification product.

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